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Ecophysiological studies on the acid tolerance of *Gammarus fossarum* (KOCH)

by WERNER MEINEL, UWE MATTHIAS and SONJA ZIMMERMANN

With 9 Figures and 4 Tables in the Text

Abstract (taken from the original document)

The acidification of running waters results in an aggravating reduction of the species composition. *Gammarus fossarum* is showing a very high sensitivity against low environmental pH and becomes damaged below pH 6 in low buffered waters. In some experiments *G. fossarum* was kept in acid waters (pH 4.5, 4.0, 3.5, 3.0) with different ionic composition. It was found out that the survival rate at pH 4.5 and 4.0 could be enhanced by increasing of the sodium concentration. Other cations (K^+, Mg^{2+}, Ca^{2+}) had no significant influence. The positive effect of sodium was related to the presence of chloride. With other anions (SO_4^{2-}) the efficiency of sodium for the survival rate is smaller. Below pH 4.0 the addition of sodium could not increase the survival time. We guess that the toxicity of environmental pH till 4.0 is caused by a failure of ionic regulation, expecially by a break down of the sodium and chloride uptake. In waters with higher H^+-concer tration other mechanisms, like acidbase balance and gas echange, could be damaged

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Introduction

The acidification of surface waters has become a problem of worldwide proportion. The introduction of acid from precipitation has a particularly significant effect in weakly buffered waters and results in a drastic reduction of the aquatic biocenosis. Gammarids, which are normally present in great quantity in our domestic highland streams and which occupy a key position in the ecology as destroyers of fallen leaves and as a basic source of food for many species of fish, exhibit particularly high sensitivity to pH levels.

For example, in the Kaufung Forest (northern Hesse/southern Lower Saxony), Gammarus fossarum was encountered only down to pH 6 (MATTHIAS & MEINEL 1982; MATTHIAS 1982, 1983); on the other hand, in eastern Hesse occasional specimens were still still found down to pH values of 5.4 (BREHM & MEIJERING 1982). Obviously, different conditions affect the physiological tolerance of low pH-values depending on the catchment area. Some studies carried out on fish (PACKER & DUNSON 1970; McWILLIAMS 1980; McWILLIAMS et al. 1980; McDONALD et al. 1980; McDONALD & WOOD 1981; ULTSCH et al. 1981; McWILLIAMS 1982) have shown that a disruption of the ion regulation mechanisms is the main cause of the inability to tolerate low pH, therefore it seemed reasonable to suppose that in the case of G. fossarum as well the ion uptake mechanism could be physiologically impaired by low pH-values in the ambient medium.

So far, very few studies have been carried out on the physiological effects of low pH on invertebrates (SHAW 1960b; STOBBART 1967; WRIGHT 1975; VANGENECHTEN et al. 1979; MORGAN & McMAHON 1982). The results varied according to species and habitat.

In the present study, the influence of various ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-) on the survival rate of Gammarus fossarum (KOCH) in various pH ranges was experimentally tested. For this purpose, the animals were removed from their natural habitat and kept in the laboratory in water from their native stream, whose ionic composition was variously modified by adding salt, as well as in synthetically produced salt solutions.

Methodology

General

The animals used in the test (Gammarus fossarum) were taken from an unpolluted, neutral highland stream (Wengebach) in the Kaufung Forest of northern Hesse, and prior to the start of testing were kept for 2 days in stream water thermostatically controlled at 15°C. The animals were not fed. The plastic dishes (base area: 200 cm^2) used in the experiments contained a constant 700 mL of test solution and each was occupied by 10 gammarids. In each case 1 day before the start of the test, the water required for the experiments was adjusted to the desired pH level by adding 0.1 N H_2SO_4 and also the desired test temperature was set. The pH was adjusted once more shortly before the animals were transferred to the test containers because the buffer

capacity of the water resulted in a delayed neutralization of the added acid and thus led to a sharp increase in the pH-value.

The pH-value was checked twice to three times daily at regular intervals. During the period of the tests the given pH-value was held constant by replacing some of the water. As a rule, the pH-value fluctuated by less than ± 0.1 pH units. When these pH checks were carried out, the number of living animals was counted and the dead animals were removed.

For each test run, 3 x 10 animals were used per pH level and concentration level. In order to check the reproducibility of the results a second and sometimes a third test run was carried out.

The Wengebach, whose water was used in some of the experiments, has a low calcium content in keeping with the geological formations through which it runs (middle and lower Buntsandstein [mottled sandstone]) and its content of relevant ions during the period of the testing is given in Table 1. The natural pH-value of the Wengebach is in the neutral range; in the period of the tests the pH-values fluctuated between 7.0 and 7.6. The mean conductivity was approximately 150 µS.

Stream water as the test medium

In order to obtain a general overview of the sensitivity of Gammarus fossarum to low pH-values, the effects of an increased H^+ concentration on the survival rates was investigated at pH-values of 3.5, 4.0, 4.5,

5.0 and 6.0. Because of the shifts in pH which were naturally greater in the upper pH ranges (5.0, 6.0) the pH checks were carried out 4 to 5 times per day at these levels.

In the following series of tests the concentrations of Na^+, K^+, Ca^{2+}, or Mg^{2+} were increased at pH 4.5 and 4.0 respectively by adding their corresponding chloride or sulfate salts. The concentrations of Ca^{2+} and Mg^{2+} were increased by adding 0.1, 0.5, 1.0, 5.0 and 1.0 mVal/L. The increases in the Na^+ concentrations which were selected are shown in Tables 2 and 3.

Synthetic salt solutions used as the test media (pH 4.5)

Within this section of the tests the starting solutions consisted of a "chloride series" and a "sulfate series". In the chloride series the Na^+, K^+, Ca^{2+} and Mg^{2+} were dissolved as chlorides, corresponding to their average concentration in the Wengebach, and in the sulfate series they were dissolved as sulfates, in double distilled water. It was necessary to divide the tests up into two separate series in order to be able to demonstrate the specific effects of Cl^-.

In order to determine whether the NaCl component in the chloride series was solely responsible for the survival rates, further experiments were carried out in pure NaCl solutions. In NaCl solution I the Cl^- concentrations were equivalent to those in the chloride series and in NaCl solution II the Na^+ concentration was equivalent to that in the chloride series. The exact composition of the salt solutions is shown in Table 4.

Additional experiments at pH 3.0, 3.5 and 4.0

In order to check whether increasing the Na^+ concentration also had effects on the survival times in the pH range below 4.0, further tests were carried out in pure NaCl solutions. At pH 4.0 the solutions contained 0.3, 5.0 and 10.0 mVal NaCl/L, at pH 3.5 and 3.0: 0.3, 5.0, 10.0, 15.0, 50.0 and 100.0 mVal NaCl/L.

Results

pH tolerance

Fig. 1 shows the sensitivity of Gammarus fossarum to acid pH levels in stream water. While no significant difference was observed at pH 6.0 compared with pH 7.0 even after a number of days, when the pH reached a value of 5.0 the survival rate towards the end of the test had dropped to just barely 60% and had thus entered a critical range. The process is particularly characterized by a sudden increase in the mortality in the range between pH 5.0 and 4.5. At pH 5.0 all the animals were dead after 5 days. A further sharp decrease in the survival rate occurred at pH 4.0, at which level 100% mortality occurred after just 1 day. In the pH 3.5 range the animals survived for only a few hours.

Influence of K^+, Ca^{2+} and Mg^{2+} on acid tolerance

The potassium, calcium and magnesium chlorides and sulfates were not found to have any significant effect on the survival rates at pH 4.0 and 4.5, i.e. an increase in the concentration of these salts in the stream water did not have any positive effect and in the synthetic medium their absence did not have a negative effect.

In contrast to the tests with synthetic solutions in which no differences whatever were obtained compared with the control tests, in the tests conducted in stream water slightly positive effects occurred in individual test runs, although these were not reproducible. This variability in the survival rates was observed in the tests carried out with an elevated Ca^{2+} concentration. For example, increasing the concentration by 1.0 or 0.5 mVal $CaSO_4$ per litre during the first run resulted in a shift in the 50% survival rate from approximately two to three and a half and three days respectively while during the second run a smaller increase in the survival rate was noted and absolutely no positive effect was recorded during the three subsequent runs.

Influence of Na^+ and Cl^-

The Na^+ ions appear able to moderate the H^+ ion stress, at least down to a certain pH-value. For example, increases in the concentration of Na^+ brought about significant improvements in the survival times. This effect was particularly noticeable when the Cl^- concentration was simultaneously increased. In the artificial solutions, the absence of Na^+ or a decrease in concentration resulted in a rapid drop in the survival rates.

Tests in stream water at pH 4.5

By adding 0.5 or 5 mVal NaCl/L the 50% survival rate was increased from approximately two to four and eight and a half days respectively (Fig. 2). In the range between 5 and 10 mVal NaCl/L a limit concentration was apparently exceeded because above a concentration of 10 mVal/L the survival times declined again.

For example, the 50% survival rates at 10 mVal/L were just barely 6 days and at 50 mVal/L 3 days. Similar findings were also reported by BROWN (1981) in the case of Salmo trutta in the 4.0 pH range: the survival rates again fell at the higher Na^+ concentrations used in the tests. Increasing the Na^+ concentration by adding Na_2SO_4 also produced clear positive effects which, however, were not as pronounced in character as when the Na+ concentration was increased by adding NaCl. In this case, a shift occurred in the optimum effect to a lower concentration range (Fig. 3). The "limit concentration" ranged between 1 and 5 mVal/L, and when 10 mVal/L were added the positive effects ceased (the values are similar to those of the control curve and therefore they have not been plotted here), and when the concentration was increased by 50 mVal/L the survival times actually declined relative to the reference curve ("toxic" concentration).

Tests in stream water at pH 4.0

The results yielded by this pH level exhibit similar tendencies to those recorded at pH 4.5. However, a larger addition (10 mVal/L) of NaCl was needed to bring about the optimum increase in the Na^+ concentration by means of NaCl (Fig. 4).

Increasing the concentration by 1.0 mVal Na_2SO_4/L also produced a slight increase in the survival rate (Fig. 5). However, when 5 or 10 mVal/L was added no further increase in the survival rate was achieved.

Tests in synthetic salt solutions at pH 4.5

For these experiments Table 4 provides an overview of the 50% survival rates at pH 4.5 as a function of the NaCl or Na_2SO_4 concentration.

Similar to the experiments conducted in stream water at pH 4.5, a 100% mortality rate was recorded in the chloride series after approximately six and a half days. On the other hand, much shorter survival times were achieved in the sulfate series; all the animals were dead after just two and a half days (Fig. 6). When the Na^+ concentration was reduced by 50%, the survival time was significantly shortened both in the chloride as well as in the sulfate series, although this effect was much more pronounced in the chloride series (cf. Figs. 6 and 7). In the absence of Na^+ the survival rates were the same in both series. In this case, 100% mortality occurred after 1 day (Fig. 8).

In NaCl solution I (NaCl concentration = 1.74 mVal/L, i.e. the same Cl^- concentration as in the entire chloride series) the 50% survival rate was situated at approximately 5 days (Fig. 9) and thus considerably higher than that achieved in the chloride series (approximately 3 days). It thus corresponded to the survival rate recorded in stream water at the same pH-value and a similar NaCl concentration (1.3 mVal/L). The survival rates in NaCl solution II (Na^+ concentration equal to that in the chloride series) were almost identical to those achieved in the chloride series despite the lack of any other cations (Fig. 5, Curve a).

Tests in synthetic salt solutions at pH 3.0, 3.5 and 4.0

The survival rates achieved at a pH of 4.0 and at Na^+ concentrations of 0.3, 5 and 10 mVal/L were almost identical with those recorded in the corresponding stream water tests at the same pH level. In contrast, no significant improvement in the survival times could be achieved either at pH 3.5 or at 3.0 by increasing the concentration from 0.3 mVal NaCl/L to 5, 10, 15, 50 and 100 mVal NaCl/L. The average lifetime at all concentration levels at pH 3.5 was approximately 8.5 hours and at pH 3.0 approximately 3.5 hours.

Discussion

Possible causes of the misregulation of Na^+ at low pH

It is generally assumed that the die-off of many organisms in acidic waters is largely due to faulty regulation of the ionic regime. It has been shown in the case of many freshwater organisms that an increase in the H^+ concentration in the ambient medium has a considerable disruptive effect on the Na^+ balance. With a declining pH-value the Na^+ loss rate increases while the rate of uptake decreases, i.e. the Na^+ concentration in the blood declines. This was demonstrated by, among others, PACKER & DUNSON (1970) for *Salvelinus fontinalis*, McDONALD & WOOD (1981) for *Salmo gairdneri*, ULTSCH et al. (1981) for *Cyprinus carpio* and McWILLIAMS (1980) for *Salmo trutta*. The Na^+ uptake may have been inhibited in the following manner:

- by competition between Na^+ and H^+ for carrier binding sides, particularly at low Na^+ concentrations;
- by an increased workload of the exchange system. Assuming that H^+ also serves as a counterion for the Na^+, in an acid milieu it has to be emitted against a steep concentration gradient. In addition, a shift in the gill potential in a more positive direction could reduce the efficiency of the uptake system.
- by the direct influence of H^+ ions on the metabolism of the Na^+-transporting cells, with a consequent reduction in the metabolic rate.
- by a blockage of the carrier system caused by a modification of its "substrate specific side".

Furthermore, the Cl^- uptake is also inhibited by a low pH-value; McDONALD & WOOD (1981) in the case of Salmo gairdneri and ULTSCH et al. (1981) in the case of Cyprinus carpio detected an increasing decline in the Cl^- content of the plasma with a declining pH-value.

Both the drop in the Na^+ plasma concentration and also in the Cl^- plasma concentration are due to a failure of the Na^+/H^+ or NH_4^+ and Cl^-/HCO_3 exchange mechanism in the gills.

Effects of increased Na^+ and Cl^- concentrations as a function of the H^+ concentration

Theoretically it can be expected that by increasing the external Na^+ concentration the conditions for the Na^+ uptake and thus also for the Na^+ balance will be improved. McWILLIAMS (1980) demonstrated this

effect in Salmo trutta; when the Na^+ concentration was increased from 30 µVal/L to 450 µVal/L at pH 4.0 he recorded an increase in the Na^+ uptake rate from approximately 0.2 to 0.75 µVal/100 g/h. Compared with an uptake rate of 5.2 µVal/100 g/h at pH 6.5 (Na^+ = 30 µVal/L) this rate was still considerably below the normal standard; nevertheless, assuming that inhibition of Na^+ regulation is a decisive factor in the death of the organisms in an acid milieu, the survival time should be improved by a high Na^+ content in the ambient medium.

This hypothesis is reinforced by the results of the present study: at a pH of 4.5 the 50% survival rate in stream water quadrupled when the Na^+ concentration was optimally increased by adding NaCl from 0.3 to 5.3 mVal/L (Fig. 2), while only a 2.5-fold increase in the survival rate occurred when the concentration of Na^+ was increased from 0.3 to 1.3 mVal/L by adding Na_2SO_4 (Fig. 3). This difference is caused simply by the additional effect of the Cl^- because the SO_4^{2-}, quite apart from its irrelevance in the physiological context, is largely impermeable.

The positive effect of the Cl^- shows up particularly clearly when a comparison is made between the survival rates of the animals in the sulfate series and the chloride series (Fig. 6), but it seems to be dependent on the presence of Na^+. For example, the survival rates in the chloride series coincide with those in the sulfate series if the Na^+ salts are missing in both series (Fig. 8). Even increasing the Cl^- concentration (stream water) by adding chlorides such as $MgCl_2$ did not bring about any lengthening of the survival times. The positive effects

of the Cl^- in the presence of Na^+ must, for the most part, be seen in conjunction with the Na^+ uptake which is increased by the Cl^-. In addition, however, it must be remembered that the Cl^- uptake is also increased in the presence of Na^+.

Since in general the internal concentration both of the Na^+ as well as of the Cl^- declines at low pH values, both uptake mechanisms are disrupted and an increased loss-rate, at least in the case of Na^+, has an additional negative effect. We can assume on the basis of the ionic regulation model that initially a certain Cl^- uptake can still take place in the absence of Na^+ or at very low Na^+ concentrations in the medium; however, in the end it must be assumed that the uptake mechanism fails because the counterion HCO_3^- is no longer available in adequate amounts as a result of the build-up of metabolic H^+ and of H^+ ions passively streaming in from the ambient medium. Therefore, it is unlikely that any lifetime-extending effect can be achieved by increasing the Cl^- concentration under these conditions alone.

The increase in the NaCl content had less of an effect at pH 4.0 than at pH 4.5. In this case the 50% survival rate could only be tripled by adding NaCl and adding Na_2SO_4 increased it only by 1/3 (Figs. 4 and 5). Absolutely no lifetime-extending effect was detected by increasing the NaCl concentration at pH levels 3.5 and 3.0.

Similar findings were reported in fish. BROWN (1981), for example, discovered in Salmo trutta that the addition of ions resulted in an increase in the survival rate only at > pH 4.0. At pH values around

4.0 not only is the ion uptake mechanism disrupted in fish but also a shift occurs in the internal acid-base equilibrium thus leading to a lethal blood-acidosis (MORGAN & McMAHON 1982; BEAMISH 1976). If the H^+ concentration continues to rise, increasing inhibition of the gas exchange most probably takes on great significance and it then results in a general tissue-hypoxemia which is the primary cause of death (PACKER 1976; ULTSCH et al. 1981).

In the case of invertebrates as well it can be assumed that at pH values around 4.0 and below, acidosis of the hemolymph and hypoxemia are increasingly superimposed on the disruption of the ionic regulation so that finally no increase in the survival rate can be achieved any longer by adding ions at very low pH values.

Influence of other cations

In the present studies, K^+ and Mg^{2+} were not shown to have any effect on the pH tolerance of Gammarus fossarum. A slightly positive effect was noted only in the case of Ca^{2+} in some tests.

At pH 4.0 and elevated K^+ and Mg^{2+} concentrations BROWN (1981) also failed to detect any increase in the survival times of Salmo trutta. There is general agreement that Ca^{2+} can moderate the stress generated by an elevated H^+ concentration, at least under certain conditions. Experiments carried out with young trout bring out a certain ambivalence in the effect of Ca^{2+}: at very low pH values (3.0, 3.2) Ca^{2+} lengthened the survival times, however at more moderate pH values (3.8, 4.4) it shortened them (McDONALD et al. 1980). On the other

hand, in the case of adult trout (Salmo trutta), the survival rate was increased by adding $CaCl_2$ at the pH levels 3.5, 3.75 and 4.0 used in the tests (BROWN 1981).

Summary

Laboratory experiments were conducted to determine the influence of the ionic concentration of the medium on the pH tolerance of Gammarus fossarum (KOCH). In order to obtain the required H^+ concentrations the test medium was acidified with 0.1 N H_2SO_4. The test temperature was 15°C.

In the first part of the tests the water, like the test animals themselves, was taken from the Wengebach, and unpolluted highland stream in the Kaufung Forest. The natural, low ionic concentration was increased by adding one of the following salts: NaCl, Na_2SO_4, KCl, K_2SO_4, $CaCl_2$, $CaSO_4$, $MgCl_2$ and $MgSO_4$ in order to determine their effects on the survival rate of the animals at pH 4.0 and 4.5.

Further experiments were carried out in synthetic salt solutions at pH level 4.5. In these experiments the Na^+, K^+, Ca^{2+} and Mg^{2+} salts were dissolved in double distilled water in concentrations corresponding to their natural concentration in the Wengebach, on the one hand in the form of chlorides in a "chloride series" and on the other in the form of sulfates in a "sulfate series". By leaving out in each case one of the salts in both series we attempted to obtain indirect evidence of the possible influence exerted by these ions on the survival rates. In

order to check whether NaCl by itself in the chloride series had any effect on the survival times of G. fossarum, further tests were carried out in pure NaCl solutions (pH 4.5). In addition, the highly toxic pH ranges of 4.0, 3.5 and 3.0 in NaCl solutions were studied in order to determine the action spectrum of this salt.

Summarizing it can be said that the Na^+, particularly in combination with Cl^-, is of vital importance for the pH tolerance of G. fossarum. As a rule, the additions of K^+, Mg^{2+} and Ca^{2+} salts did not produce any significant deviations from the control. Their absence from one test series using pure NaCl solution had no negative effect.

In our stream water tests the best results were achieved by adding NaCl. The 50% survival rate was increased by a factor of four at pH 4.5, namely from slightly more than two to eight and a half days; in contrast, when Na_2SO_4 was added the survival rate increased only to 5 days. At pH 4.0 these effects were no longer pronounced. At the optimum NaCl concentration the 50% survival rate at pH 4.0 was 45 hours compared with 16 hours in the control test; the most effective addition of Na_2SO_4 was only able to increase the survival rate to 21 hours.

In a test series using "synthetic" salt solutions with the same cation concentration as that found in stream water, where the chloride functioned as an anion, similar survival rates were achieved to those obtained in the stream water control tests. In contrast, in another test series using the same concentration of cations with sulfate as the counterion, only 50% of the animals were still alive after just barely

one and a half days. In both the sulfate and the chloride test series, leaving out the Na^+ resulted in an identical 50% survival rate of 18 hours. This result would indicate that the Na^+ and Cl^- effects are to some extent dependent on each other; the Cl^- seems to affect the pH tolerance only in the presence of Na^+.

At pH 3.0 and 3.5 - in contrast to the results obtained at pH 4.0 and 4.5 - no effects on the survival times were noted by increasing the NaCl concentration.

The results obtained indicate that the physiological stress to which the animals are exposed by the increased H^+ concentration can be considerably moderated, at least at pH values of 4.0 and up, by adding Na^+ and Cl^- in certain concentrations.

Taking account of the Na^+ and Cl^- uptake conditions and ionic regulation models, the paper discusses the possible physiological effects of an elevated H^+ concentration on Gammarus fossarum. Three principal disruptive factors were considered:

1. failure of the ionic regulation mechanism,
2. shifts in the acid-base equilibrium, which are closely correlated with the inhibition of ion uptake, and
3. disruptions in the gas exchange.

For some species it is assumed that the failure of the ionic regulation mechanism and the shifts in the acid-base equilibrium are the primary causes of death in the pH range around and above pH 4.0.

In the pH range below 4.0 the decisive role is probably played by disruptions in the gas exchange process. The results obtained from our studies on G. fossarum also point in this direction.

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See following page for translation of foreign titles

Translation of foreign-language bibliography titles

1. On the sensitivity of selected freshwater crustaceans (*Daphnia* and *Gammarus*) to acid stress.

2. On the anthropogenic acidification of water in the Kaufung Forest.

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Table 1: Average values of some chemical parameters in the Wengebach and the analytical methods employed

	mg/l	mVal/l	Analysenverfahren (1)	
Na^+	6,75	0,29	Flammenspektrometrische Bestimmung (ZEISS PF 5)	(2)
K^+	2,76	0,07	Flammenspektrometrische Bestimmung (ZEISS PF 5)	(2)
Ca^{2+}	13,50	1,10	Maßanalytisch mittels ÄDTA (Titriplex III)	(3)
Mg^{2+}	5,37	0,27	Maßanalytisch mittels ÄDTA (Titriplex III)	(3)
Cl^-	10,50	0,30	Maßanalytische Bestimmung	(4)

Translation key:

1. Analytical method
2. Flame spectrometry
3. Titrimetric analysis, using ethylene diamine tetraacetate (Titriplex III)
4. Titrimetic analysis

Table 2: 50% survival rate at pH 4.0 and various Na^+ concentrations

	① Salzzugabe in mVal/l	② 50%ige Überlebensrate nach x Stunden
③ Bezugskurve	–	50
④ Zugabe von NaCl	0,5	102
	1,0	120
	5,0	206
	10,0	139
	50,0	72
⑤ Zugabe von Na_2SO_4	0,5	113
	1,0	127
	5,0	74
	10,0	48

Translation key:
1. Salt added, in mVal/L
2. 50% survival rate after x hours
3. Reference curve
4. Addition of NaCl
5. Addition of Na_2So_4

Table 3: 50% survival rate at pH 4.0 and various Na^+ concentrations

	① Salzzugabe in mVal/l	② 50%ige Überlebensrate nach n Stunden
③ Bezugskurve	–	16
④ Zugabe von NaCl	5,0	37
	10,0	45
	50,0	27
⑤ Zugabe von Na_2SO_4	1,0	21
	5,0	16
	10,0	15

Translation key:
1. Salt added, in mVal/L
2. 50% survival rate after n hours
3. Reference curve
4. Addition of NaCl
5. Addition of Na_2So_4

Table 4: 50% survival rate in differently constituted test media, pH 4.5

	(1) 50%ige Überlebensrate nach x Stunden	(2) Konzentration in mVal/l					
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}
(3) A **Chloridreihe**	70	0,29	0,07	1,10	0,27	1,74	–
B Chloridreihe mit halber (4) NaCl-Konzentration	50	0,15	0,07	1,10	0,27	1,60	–
C (5) Chloridreihe ohne NaCl	18	–	0,07	1,10	0,27	1,45	–
(6) NaCl-Lösung I	125	1,74	–	–	–	1,74	–
(6) NaCl-Lösung II	72	0,29	–	–	–	0,29	–
a (7) **Sulfatreihe**	31	0,29	0,07	1,10	0,27	–	1,74
b Sulfatreihe mit halber (8) Na_2SO_4-Konzentration	24	0,15	0,07	1,10	0,27	–	1,60
c (9) Sulfatreihe ohne Na_2SO_4	18	–	0,07	1,10	0,27	–	1,45

Translation key:

1. 50% survival rate after x hours
2. Concentration mVal/L
3. Chloride series
4. Chloride series with half the NaCl concentration
5. Chloride series without NaCl
6. NaCl solution
7. Sulfate series
8. Sulfate series with half the Na_2SO_4 concentration
9. Sulfate series without Na_2SO_4

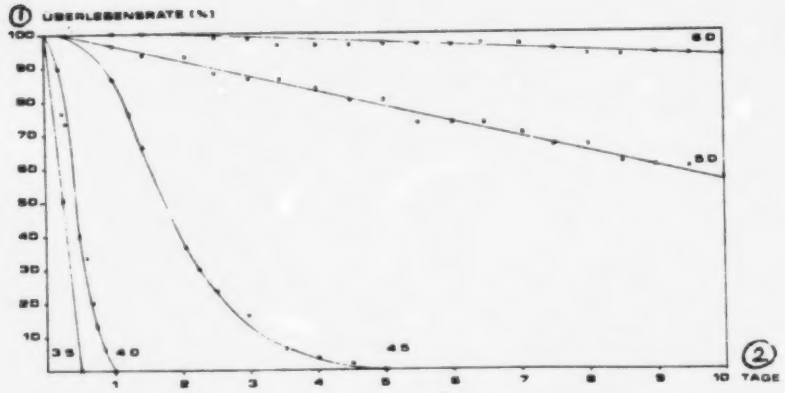

Fig. 1: Survival rates of G. fossarum at various pH values in stream water

Translation key:
1. Survival rate
2. Days

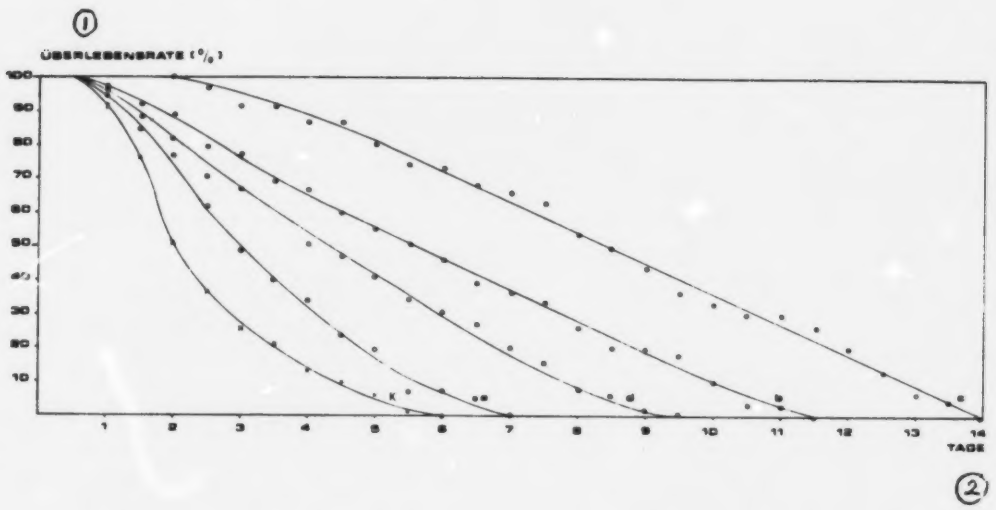

Fig. 2: Survival rates of G. fossarum with various additions of NaCl to the stream water at pH 4.5 (a = 50 mVal/L, b = 10 mVal/L, c = 5 mVal/L, d = 0.5 mVal/L, K = no NaCl added)

Translation key:
1. Survival rate
2. Days

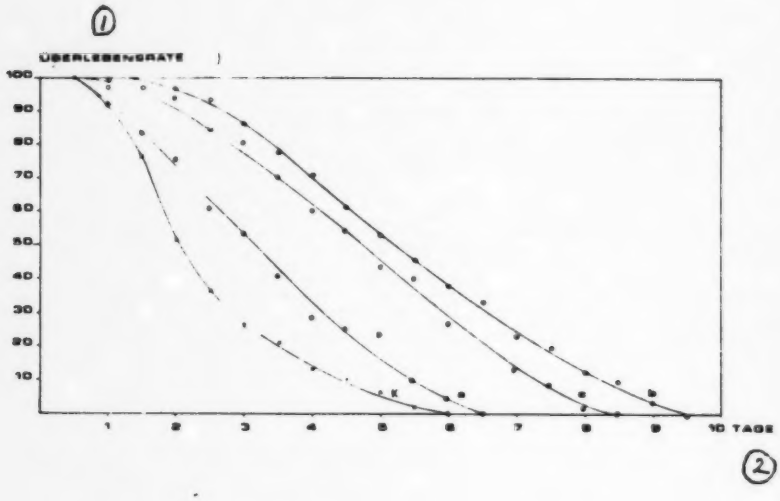

Fig. 3: Survival rates of G. fossarum with various additions of Na_2SO_4 to the stream water at pH 4.5 (a = 5 mVal/L, b = 1 mVal/L, c = 0.5 mVal/L, K = no Na_2SO_4 added)

Translation key:
1. Survival rate
2. Days

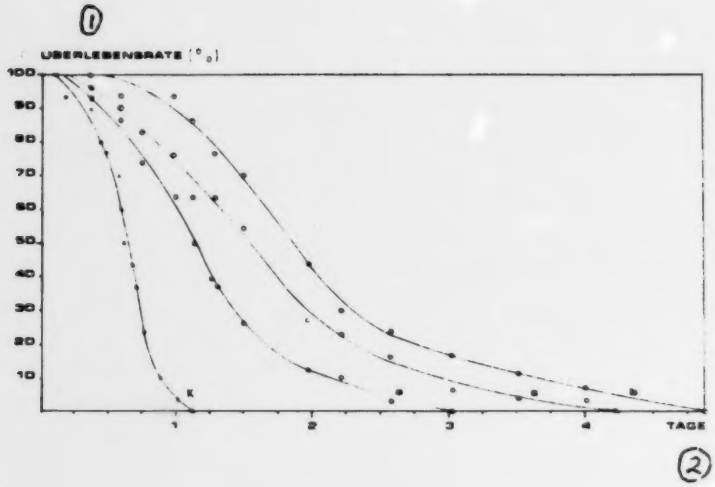

Fig. 4: Survival rates of G. fossarum with various additions of NaCl to the stream water at pH 4.5 (a = 50 mVal/L, b = 10 mVal/L, c = 5 mVal/L, K = no NaCl added)

Translation key:
1. Survival rate
2. Days

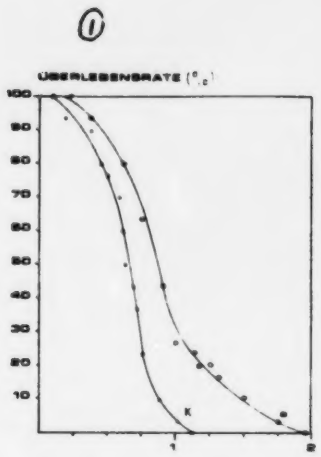

Fig. 5: Survival rates of G. fossarum with various additions of Na_2SO_4 to stream water at pH 4.0 (a = 1 mVal/L, K = no NaCl added)

Translation key:
1. Survival rate

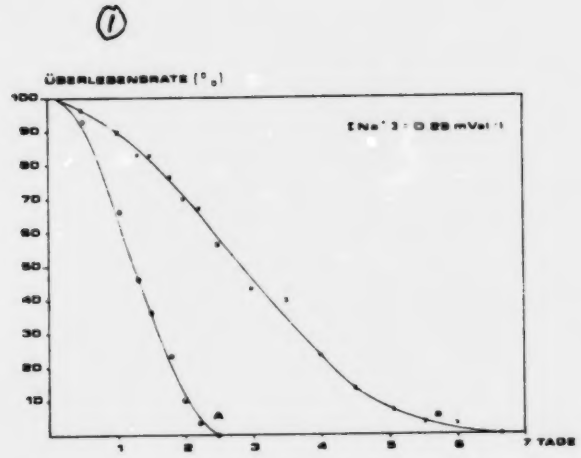

Fig. 6: Survival rates of G. fossarum in a synthetic salt solution with 0.29 mVal Na^+/L at pH 4.5 (A = chloride as the anion, a = sulfate as the anion)

Translation key:
1. Survival rate

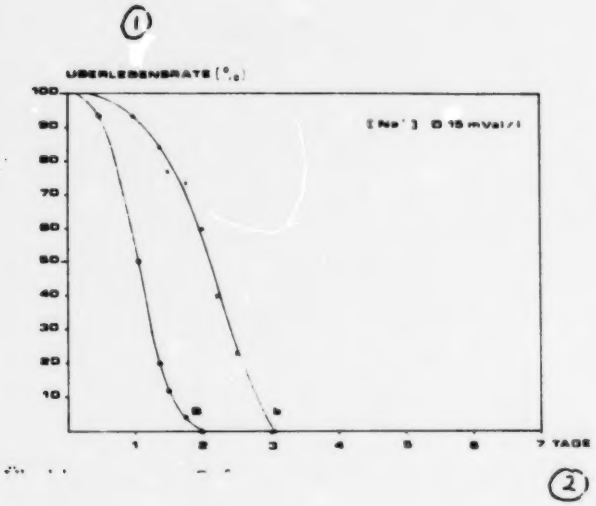

Fig. 7: Survival rates of G. fossarum in a synthetic salt solution with 0.15 mVal Na^+/L at pH 4.5 (B = chloride as the anion, b = sulfate as the anion)

Translation key:
1. Survival rate
2. Days

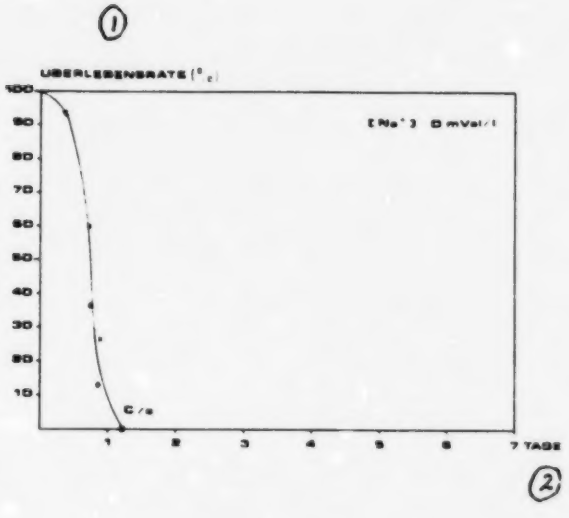

Fig. 8: Survival rates of G. fossarum in a sodium-free salt solution at pH 4.5 (C = chloride as the anion, c = sulfate as the anion)

Translation key:
1. Survival rate

2. Days

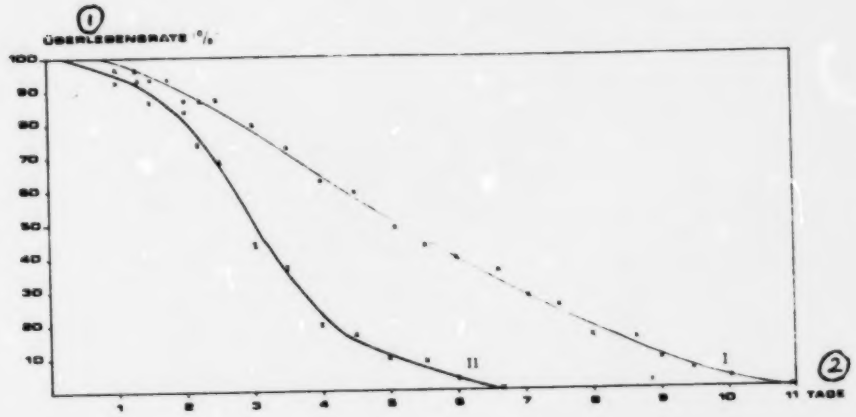

Fig. 9: Survival rates of G. fossarum in a pure NaCl solution at pH 4.5 (I = 1.74 mVal/L, II = 0.29 mVal/L)fate as the anion)

Translation key:
1. Survival rate

2. Days